

Antiandrogenic Effects of a Phthalate Combination on *In Utero* Male Reproductive Development in the Sprague-Dawley Rat: Additivity of Response?

P.M.D. FOSTER, K.J. TURNER¹ and N.J. BARLOW^{1,2}

¹CIT Centers for Health Research, Research Triangle Park, North Carolina 27709
²North Carolina State University, Raleigh, North Carolina 27606

Abstract

Environmental antiandrogens constitute a class of chemicals that significantly affect reproductive development in laboratory animals. In the environment it is more likely that exposure will occur to multiple agents that may have similar or different mechanisms of antiandrogenicity. The objective of the present study was to examine the reproductive developmental effects on male rats exposed to two phthalates *in utero*, both as individual compounds and in combination. Dose levels were selected based on LOAELs for reproductive development obtained from previous dose-response studies. Pregnant Crl:CD(SD)BR rats, 10 per group, were treated by gavage with either corn oil vehicle, 100 mg/kg/day di(n-butyl) phthalate (DBP) or di(2-ethylhexyl) phthalate (DEHP) or a combination of each phthalate at 100 mg/kg/day (effectively 200 mg/kg/day of phthalate) on gestational days 12 to 21. On postnatal day (PND) 1 the anogenital distance (AGD) and male pup body weights were measured and each individual pup was uniquely identified with a footpad tattoo. On PND 13 the retained areola were counted on each male pup. At necropsy on PND 90 the AGD was again measured and the rats were shaved to reveal permanent nipples. Organ weights were recorded for the testes, epididymides, prostate (dorsolateral and ventral) and seminal vesicles. On PND 1 the AGD was significantly reduced in the DBP and combination groups while it did not differ from control for DEHP alone. Significantly increased numbers of areola were retained on PND 13 in the males in the DBP and combination groups but not following DEHP exposure. There were no consistent changes in organ weights and only a small number of reproductive tract lesions were seen in the phthalate-deficient group. The results of this study do not support the definitive assumptions that the effects of prenatal exposure to a combination of two phthalates on AGD and nipple retention in male rats is additive in comparison with the effect of exposure to each phthalate alone.

Background

The phthalates DBP and DEHP have been shown to act as antiandrogens when males are exposed to them.

They are thought to have a common mode of action, decreased testosterone production by the fetal testes.

It is unknown if these compounds, administered in combination, will produce additive effects on male reproductive development.

The Food Quality Protection Act of 1996 states that the risk from endocrine active compounds acting via the same mechanism should be aggregated.

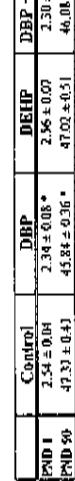
This study experimentally tests whether it is appropriate to aggregate risk from compounds that have similar mechanisms of action.

The doses of DBP and DEHP were chosen to approximate LOELs for each of the compounds.

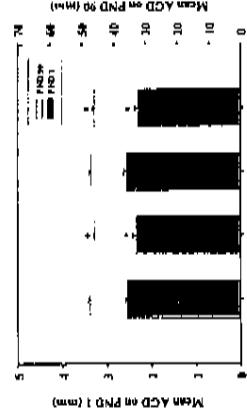
Hypothesis

In vitro exposure to a combination of low doses of DBP and DEHP will induce additive effects on *in utero*-dependent reproductive development. The effects of exposure to a combination of phthalates on AGD, areola / nipple retention and the incidence of male reproductive tract malformations will exceed that observed when each phthalate is administered alone.

Study Design and Methods



*Significant difference between Control and DBP+DEHP groups.
†Significant difference between Control and DEHP groups.
‡Significant difference between Control and DBP groups.
§Significant difference between DBP and DEHP groups.



*Significant difference between Control and DBP+DEHP groups.
†Significant difference between Control and DEHP groups.
‡Significant difference between Control and DBP groups.
§Significant difference between DBP and DEHP groups.

Dam Reproductive Data

Sperm (ml) / day	Control				DBP		DEHP		DBP + DEHP	
	Frequent	10	10	10	10	10	10	10	10	10
Female Weight (g) ^a	107 ± 5	108 ± 5	109 ± 5	109 ± 5	108 ± 5	107 ± 5	107 ± 5	108 ± 5	107 ± 5	107 ± 5
GD 0	197 ± 5	257 ± 5	257 ± 5	257 ± 5	257 ± 5	257 ± 5	257 ± 5	257 ± 5	257 ± 5	257 ± 5
GD 9	261 ± 5	284 ± 6	279 ± 6	279 ± 6	279 ± 6	279 ± 6	279 ± 6	279 ± 6	279 ± 6	279 ± 6
GD 12	284 ± 6	319 ± 6	319 ± 6	319 ± 6	319 ± 6	319 ± 6	319 ± 6	319 ± 6	319 ± 6	319 ± 6
GD 21	383 ± 7	378 ± 7	391 ± 7	391 ± 7	386 ± 6	386 ± 6	386 ± 6	386 ± 6	386 ± 6	386 ± 6
PND 21	513 ± 6	514 ± 6	514 ± 6	514 ± 6	512 ± 6	512 ± 6	512 ± 6	512 ± 6	512 ± 6	512 ± 6
Weight gain (g) ^a	1012 ± 4	992 ± 4	1122 ± 4	1122 ± 4	1122 ± 4	1122 ± 4	1122 ± 4	1122 ± 4	1122 ± 4	1122 ± 4
GD 12 to GD 21	1012 ± 4	992 ± 4	1122 ± 4	1122 ± 4	1122 ± 4	1122 ± 4	1122 ± 4	1122 ± 4	1122 ± 4	1122 ± 4

^aTwo dams lost one pup; one female was collared (not included).
*Significant difference between Control and DBP+DEHP groups.

No differences in body weight or body weight gain were observed during the closing of lactation period.

The data in the table represents lesions consistent with an androgenic toxic mechanism including atrophic and enlarged testes, hypoplastic epididymides and lack of attachment of the tail of the epididymides to the scutum.

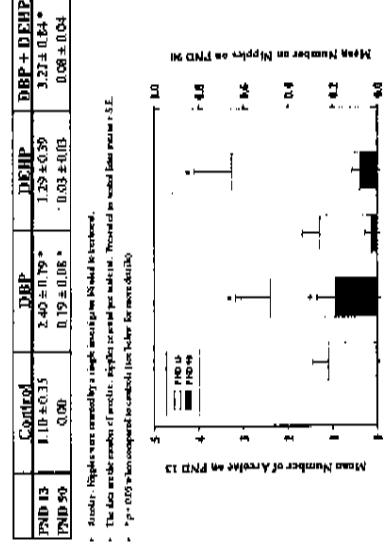
Each animal with a lesion, regardless of the number of lesions or organs affected, was treated as a single affected animal.

The DEHP group had more affected animals and litters and appears to predominantly contribute to the lesions seen in the combination group.

Histopathology

- The incidence of histologic lesions was less than that of gross lesions.
- Three animals, one in the DEHP group and two in the combination group, had histologic lesions corresponding to gross lesions.
- No histologic lesions were observed in the absence of gross lesions.

Male Areolae / Nipple Retention



*Significant difference between Control and DBP+DEHP groups.
†Significant difference between Control and DEHP groups.
‡Significant difference between Control and DBP groups.
§Significant difference between DBP and DEHP groups.

- Stress. Pups were exposed to a single dose of DEHP for 1 hour prior to weaning.
- The dose was the random of control. Pups were exposed to a single dose of DEHP for 1 hour prior to weaning.
- * p < 0.05 vs control (p-value for analysis of variance).
- † p < 0.05 vs control (p-value for analysis of variance).

Summary

- The areolae and nipple data was analyzed similarly to the AGD data through Dunn's one-tailed test was based on the *a priori* hypothesis that the areole / nipple counts would increase when an animal is exposed to an antiandrogen *in utero*.
- A 2 x 2 factorial analysis of the data indicated that DEHP at 100 mg/kg/day on PND 12 to 21 had a significant effect on areole / nipple retention while DEHP did not. The effect of PND 13 was observed in both the DEHP and combination groups while all PND 90 occurred only in the DEHP group.
- The significant effect on areole / nipple retention observed in the combination group was predominantly due to exposure to DEHP in the combination with no statistical interaction observed between the two compounds.
- Doses of 100 mg/kg/day of DBP at 100 mg/kg/day on PND 12 to 21 did not affect dam reproductive parameters.
- AGD was significantly and permanently reduced when mice were exposed to DBP or a combination of phthalates *in utero*. However, the reduction in the combination group was due to the presence of DEHP and not DEHP.
- Areola retention was increased with gestational DBP or phthalate combination exposure. While this increase was not a permanent effect in the combination group, the increase observed in the combination group at PND 13 was predominantly due to the effect of DBP exposure.
- The explanation for the lack of DEHP effects on AGD and areole / nipple retention was likely that the compounds were given at equal doses, which is not equimolar, and therefore the dams were effectively receiving less DEHP than DBP.
- This study did not indicate an additivity of response or an interaction of the two phthalates in combination. Aggregation of risk at these doses would not be appropriate.

Acknowledgements

- The authors would like to acknowledge Dr. Earl Gray of the United States EPA for his assistance in the statistical analysis and interpretation of the data. We would also like to thank the staff in the Histopathology and the Animal Care Unit at the CIT Centers for Health Research for their contributions to this study. Assistance from colleagues in the System Biology Division was also greatly appreciated.